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Real-time detection of gas-producing bacteria in milk by Raman spectroscopy

Gas-producing bacteria can compromise dairy product quality and cause significant economic losses. In particular, *Clostridium* contamination may induce late-blowing defects in hard and semi-hard cheeses through hydrogen and carbon dioxide production during ripening. Rapid detection methods are therefore highly desirable. A Raman spectroscopy-based system was developed for real-time monitoring of hydrogen and carbon dioxide in the headspace of vials within the Most Probable Number (MPN) framework [1]. Raman spectra are converted into gas partial pressures through calibration with gas mixtures of known hydrogen and carbon dioxide concentrations. A vial is classified as positive when the measured gas signal exceeds a threshold defined as the mean background signal during the initial incubation phase plus five standard deviations. This approach enables quantitative monitoring of gas accumulation and objective assessment of positivity. Figure 1 shows representative hydrogen and carbon dioxide evolution curves during *Clostridium* incubation, with six positive vials out of nine analyzed. Current experiments on raw milk samples collected immediately after milking and inoculated with *Clostridium*, *Bacillus licheniformis* and *Anaerocolumna aminovalerica* are evaluating the potential of the system to both detect and discriminate gas-producing bacteria under realistic dairy production conditions.

References

- [1] D. Barbiero, et al. "Fast detection of *Clostridia* contamination in milk by Raman spectroscopy", Proc. SPIE 14086, Optical Sensing and Detection IX, 140861F (29 May 2026); <https://doi.org/10.1117/12.3100013>

Figures

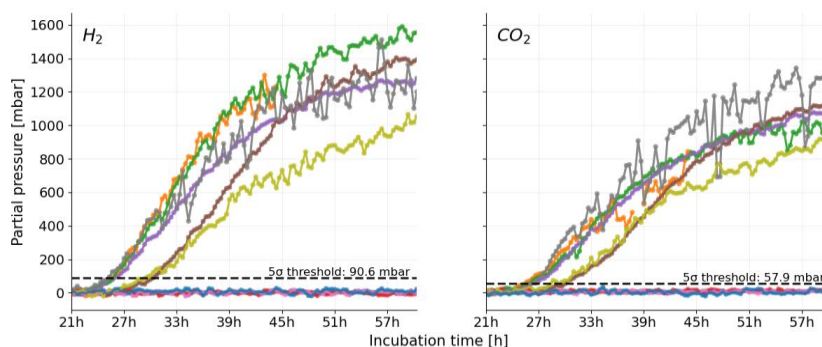


Figure 1: Temporal evolution for each vial of hydrogen and carbon dioxide partial pressure during *Clostridium* incubation.